User Manual: *Patterning Cells in Gelatine Methacryloyl Followed by Light Crosslinking*

Purpose

This user manual outlines the steps for cell patterning in **gelatine methacryloyl (GelMA)** by use of mimiX labware and cymatiX. It provides guidance for standard cell patterning and highlights the most important DOs and DON'Ts. Please adjust according to your needs and experimental goals.

Additional user manuals needed:

- *Frame Preparation and Patterning Parameters*

1. Description of GelMA Hydrogels

GelMA can be ordered from Sigma at a Bloom strength of 300 and a substitution of 40%.

https://www.sigmaaldrich.com/CH/en/product/aldrich/900629?context=product

Other products at higher substitution or sterile filtered GelMA solutions are also available, but not detailed in this protocol.

GelMA bears methacryloyl sites that react upon activation with a photoinitiator, e.g. Irgacure or Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) after irradiation at 365 nm or 405 nm, respectively. Subsequently, gelatine crosslinks and forms a stable gel. Without light crosslinking, GelMA becomes solid at room temperature at concentrations of 5% (w/v) and higher which can be reversed by placing it at 37°C. GelMA is a natural product and batch to batch variabilities occur. Please always check the data sheet provided with each delivery.

Patterning with cymatiX works optimal at GelMA concentrations of 2 % (w/v) or 5% (w/v). This leads to relatively soft, but stable gels. At higher concentrations, the increased viscosity impairs patterning of single cells. An optimal concentration needs to be established for each individual experiment and is dependent on the cell type, cell size (spheroids or single cells) and the intended research goal. If patterned at elevated temperatures, 37°C, the viscosity is reduced.

Table 1.1 *Chemicals needed for GelMA hydrogels*

Item #	Name	Catalogue #	Storage	Distributor
1	GelMA, lyophilised	900629-1G	4°C, protected from light, under Argon	Sigma
2	Irgacure 2959, with lamp at 365 nm	410896-10G	RT, protected from light	Sigma
3	LAP, with lamp at 365 or 405 nm	900889-1G	4°C, protected from light	Sigma
4	Methanol, if working with Irgacure	179337-500ML	RT	Sigma

1.2 Additional chemicals and materials needed

- Expanded cells, depending on the experiment
- General cell lab consumables
- mimiX frames
- PBS to wet frames, see user manual *Frame Preparation and Patterning Parameters* for details.
- Sterile tweezers to assemble frames
- Reaction vials
- Petri dishes from Techno Plastic Products AG, Switzerland (www.tpp.ch), 60 mm in diameter, catalogue number 93060

2. Preliminary checks and preparation

- If needed, sterilise labware and instruments prior to use
- Calculate the needed amount of chemicals according to table 3.1
- Start cymatiX and perform the initialisation
- To work with GelMA, the UV-unit on cymatiX is needed

3. Procedure

3.1 Frame preparation and patterning parameters

If you are a first-time user of cymatiX and mimiX labware, please carefully read the user manual *Frame Preparation and Patterning Parameters.*

3.2 Compound reconstitution and hydrogel preparation

For further information and certificates, please see the manufacturer's homepage

https://www.sigmaaldrich.com/CH/en/product/aldrich/900629?context=product

Volumes in table 3.1 are given for a S10 frame of 80 µL. A working solution of 10 % (w/v) is prepared first. (Note: in this case, weight per volume is defined as weight of GelMA in volume of PBS, not total volume of the solution). This solution can be stored at 4°C for a few days but should not be used after 1 week since crosslinking can be impaired. Irgacure or LAP solutions can be kept for 2 weeks, stored at 4°C and protected from light. Avoid preparing large amounts.

Please note that the cell suspension will be diluted by a factor of 5, thus prepare a 5x concentrated cell suspension. For example, a final density of $1 \cdot 10^6$ cells per mL has been successfully used for a variety of experiments; accordingly, an initial cell suspension of $5 \cdot 10^6$ cells per mL was prepared.

Stock solution preparation

Preparing stock solutions allows for easier adjustment of experiment-specific concentrations.

GelMA
GelMA lyophilisates can be sterilised with Ethylene Oxide (to be sent to specialised companies). Prepare a GelMA stock solution of 10 % (w/v) by dissolving 50 mg GelMA in 500 µL PBS. Dissolve GelMA under gentle stirring at 37°C in an incubator or water bath. GelMA stock solutions can be stored at 4°C for a few days. Liquefy at 37°C prior to use.

Irgacure (if working with irradiation at 365 nm)
Prepare an Irgacure stock solution of 10 % (w/v) by dissolving 25 mg Irgacure in 250 µL Methanol. Wrap vial in tin foil and store at 4°C protected from light. Stock solutions can be stored for two weeks. Sterile filter prior to use.

LAP (if working with irradiation at 365 or 405 nm)
Prepare a LAP stock solution of 2.5 % (w/v) by dissolving 25 mg LAP in 1000 µL PBS. Wrap vial in tin foil and store at 4°C protected from light. Stock solutions can be stored for two weeks. Sterile filter prior to use.

Table 3.1 *Protocol to prepare 85 µL GelMA hydrogel (with Irgacure) to pattern cells in a S10 frame of 2 % or 5 % (w/v) final GelMA concentration. Volumes of the respective component are given in µL.*

	Final GelMa concentration	
Component	2% (w/v)	5% (w/v)
GelMA working solution (µL)	17	42.5
Irgacure Stock Solution (µL)	0.85	0.85
Cell suspension, 5x (µL)	17	17
PBS (µL)	50.15	24.65

Table 3.2 *Protocol to prepare 85 µL GelMA hydrogel (with LAP) to pattern cells in a S10 frame of 2 % or 5 % (w/v) final GelMA concentration. Volumes of the respective component are given in µL.*

	Final GelMa concentration	
Component	2% (w/v)	5% (w/v)
GelMA working solution (µL)	17	42.5
LAP Stock Solution (µL)	3.4	3.4
Cell suspension, 5x (µL)	17	17
PBS (µL)	47.6	22.1

- Add PBS to a reaction vial (Eppendorf tube or similar)
- Add GelMA working solution and mix well.
- Do not vortex and avoid bubble formation
- Add cell suspension
- Keep at 37°C until patterning
- Add Irgacure **or** LAP just before patterning

3.3 Patterning

Before patterning, please carefully read the user manual Frame Preparation and Patterning Parameters which provides examples of patterning parameters. For your own experiment, frequency, acceleration, and duration of the stimulation must be adjusted to address specific experimental requirements and the intended pattern geometry.

Depending on the cell concentration, cell size and the chosen GelMA concentration, a pattern will be formed within 10 seconds to 2 minutes. For increased local cell density enhancement (more defined pattern), the stimulation can be applied longer.

After switching off the sound, cure GelMA at the appropriate wavelength of 365 nm (Irgacure) or 405 nm (LAP) for 3 minutes at an Intensity of 5%. Gently add medium before placing the petri dish into a humidified cell culture incubator. For a 60 mm petri dish, 6 mL medium are sufficient to cover the sample. Gently add the medium along the side of the petri dish. It is important that the gel is fully formed prior to medium addition.

Your cell pattern is now ready to be cultured and analysed.

4. Appendix

4.1 Examples and Figures

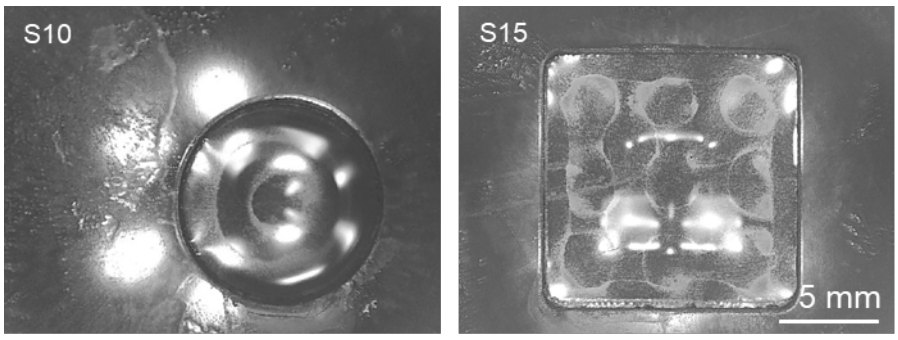

Figure 1 *Cells were patterned in 2% (w/v) GelMA in mimiX frames of series S10 or S15. In the larger frame, more complex patterns can be produced.*

4.2 Special Hints

- For a prolonged patterning experience and time to gelation, the petri dish with the frame can be warmed (placed in the incubator) prior to use.
- If the gel solidifies prior to UV crosslinking, it can be warmed at 37°C to liquefy again.

4.3 Trouble Shooting Guide

Problem	Solution
10% stock solution does not become liquid	Leave longer at 37°C Contamination with Irgacure might have led to irreversible light crosslinking.
GelMA does not become solid after light crosslinking	Is the light source correctly installed? Did you choose the correct wavelength and intensity? Was the photoinitiator solution freshly prepared and protected from light? Crosslinking and final hydrogel properties might be impaired by aged solutions or changes in applied parameter.
No pattern is visible	Did you adjust the camera in order to see the cells? GelMA might have gelled before patterning, make sure it is kept protected from light. Gelation also occurs at room temperature (reversible if warmed).
The gel is too liquid at the end	Try to work at a higher concentration. 2% results in very soft gels. At higher concentration, however, patterning is harder to achieve and needs more time due to the increased viscosity.